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I LEWENSOHN-FUCHS
Z BERKE
D WESTER
T DALIANIS
Dept. of Immunology, Microbiology, Pathology, and Infectious Diseases
K ELFGREN
Dept. of Gynaecology and Obstetrics, Karolinska Institutet, Huddinge Hospital F79, 141 86 Huddinge, Sweden

Correspondence to: Dr T Dalianis.

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Ciprofloxacin resistant gonococci arriving from Thailand

In an attempt to overcome the increasing isolation of penicillin resistant gonococci (both chromosomal and plasmid-mediated), some genitourinary medicine clinics in London now use single-dose oral ciprofloxacin as first-line therapy. This is in line with the World Health Organisation's 1989 recommendations.[1] Ciprofloxacin has the advantage of costing less than spectinomycin or third-generation cephalosporins. Unfortunately, there have been recent reports of resistance to ciprofloxacin with associated clinical treatment failures in London.[2]

We report a 36 year old lorry driver who presented with uncomplicated gonococcal urethritis having just returned from a two week holiday in Thailand where he admitted to having unprotected sexual intercourse with a Thai female. He had not received antibiotics in the previous three months. Staining of his purulent urethral discharge demonstrated Gram negative intracellular diplococci. He was treated empirically with spectinomycin 2 g i.m. and was cured both clinically and microbiologically.

Neisseria gonorrhoeae isolated from the pus at 48 hours exhibited low-level resistance to penicillin (MIC 0·5 mg/l, β-lactamase negative), resistance to tetracycline (MIC 4 mg/l) and decreased susceptibility to ciprofloxacin (MIC 0·25 mg/l). The isolate was fully sensitive to cefotaxime (0·015 mg/l) and spectinomycin (32 mg/l). A growth requirement for proline and expression of the protein 1B-2 serovar were demonstrated by conventional typing.

There is an increasing trend to use oral rather than parenteral treatment for uncomplicated gonococcal infection, hence the current popularity of ciprofloxacin. Taking an accurate travel history from patients with gonorrhoea is crucial in deciding which is the most appropriate first-line agent to prescribe in order to minimise the risk of treatment failure. Gonococci with markedly reduced susceptibility to ciprofloxacin (MIC $\geqslant$2 mg/l) have been reported in studies from South-East Asia. One study from the Phillipines reported an MIC_{90} for ciprofloxacin of 0·25 mg/l[3] and another in Thailand reported 0·3% of gonococcal isolates to have a ciprofloxacin MIC $\geqslant$2 mg/l.[4] Gonorrhoea treatment failures have been associated with ciprofloxacin MICs exceeding 0·12 mg/l.[2] In Thailand, a recent study showed 9% of gonococci to be spectinomycin resistant (MIC $\geqslant$128 mg/l) whereas 100% of isolates were susceptible to cefotaxime.[4] Worldwide, resistance to broad-spectrum cephalosporins is rare. Despite the success with spectinomycin in our patient, it would be more logical for patients acquiring gonorrhoea in South-East Asia to be treated empirically with single-dose therapy using a third generation cephalosporin such as ceftriaxone (i.m.), cefotaxime (i.m.) or cefixime (oral). Spectinomycin and ciprofloxacin may be more appropriate as second-line agents. There is a need for continued antibiotic susceptibility surveillance of *N gonorrhoeae* isolates originating from the tropics in order to prevent dissemination of multi-resistant gonococci into the United Kingdom.

DA LEWIS
GE FORSTER
BT GOH
Ambrose King Centre, Royal London Hospital, Whitechapel, London E1 1BB, UK

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Suitability of *Neisseria gonorrhoeae* lipooligosaccharides for epidemiological studies

Although the lipooligosaccharides (LOSs) of *N gonorrhoeae* are multicomponent and display considerable interstrain heterogeneity,[1] the components of the LOSs of individual *N gonorrhoeae* strains have been shown to

Relationship between SDS-PAGE analysis of LOS profiles and auxotype, serotype, β-lactamase plasmids and multiple antibiotic resistance in Neisseria gonorrhoeae strains

	LOS pattern*						
	I	*I'*	*II*	*II'*	*III*	*IV*	*V*
Auxotype							
Proto	1	2	17	1	18	4	1
Prl-	1	2	11	—	12	5	2
Arg-Prl-	—	—	4	1	1	2	—
Arg-	2	—	—	—	2	—	—
Other	—	—	2	—	3	1	—
Serotype							
Art	1	—	—	—	5	1	3
Arst	—	—	2	1	5	—	—
Av	1	1	—	—	2	1	—
At	—	—	1	—	2	—	—
Other PIA	—	—	3	—	1	2	—
Brop	2	1	5	—	12	4	—
Bropt	—	—	5	—	—	—	—
Bpyvut	—	—	6	—	—	—	—
Bo	—	2	—	—	1	2	—
Broyst	—	—	4	—	—	—	—
Bopt	—	—	—	—	3	—	—
Bx	—	—	2	—	—	1	—
Other PIB	—	—	5	4	6	1	—
PPNG strains†							
Africa −	—	1	7	—	4	—	—
Asia −	—	—	—	—	4	—	—
Asia +	—	—	—	—	—	1	—
Multiple antibiotic resistant‡	—	1	12	—	3	1	1

* Molecular weight (daltons) of lipooligosaccharides profiles: I > 6000; I' 5800–6000; II' 5000; II' 4800–5000; III 4550; IV 4000 and V 3300.
† n: 17 PPNG strains.
‡n: 18 multiple resistance strains.

exhibit constant SDS-PAGE migration patterns[2 3] which, in turn, may be valuable for epidemiological tracing in gonococcal disease.

We compared conventional typing methods (auxotyping, serotyping, plasmid profile and antibiotic sensitivity testing) with the SDS-PAGE LOS profiles in the differentiation of 95 strains of *N gonorrhoeae* isolated from symptomatic untreated patients with urogenital gonococcal infection. Characterisation of LOSs of the different strains of *N gonorrhoeae* was performed by a proteinase K digest of whole-cell lysates as described by Hitchcock and Brown.[4] All studies were performed in duplicate. Briefly, the pellets from each *N gonorrhoeae* strain were solubilised in a lysing buffer containing 2% SDS (w/v), 4% 2-mercaptoethanol (v/v), 10% glycerol (v/v), 1 *M* Tris (pH 6·8), and 0·056% bromphenol blue (w/v). Lysates were heated at 100°C for 10 min. Proteinase K 250% (w/v) solubilised in the lysing buffer was used for protein digestion. LOS samples were separated through polyacrylamide gels. Electrophoresis was done at 15 mA constant current with 25 mM Tris-142 mM glycine-0·1% SDS, pH 8·3, for 6 h. Simultaneous electrophoresis with the *Salmonella minnesota* rough mutant LOSs as molecular size markers was done. After electrophoresis the gels were stained with silver according to the method of Tsai and Frasch (periodate oxidation)[5] and compared by densitometry.

Seven different electrophoretic patterns (I, I', II, II', III, IV and V) for gonococcal LOS with an estimated molecular weight between 3300 and >6000 daltons were obtained. To ensure the reproducibility of analysis of gonococcal LOS by SDS-PAGE, different loading doses were assayed. At a loading dose of 10 μg, resolution of LOS components was optimal. Patterns II and III grouped 35·8% and 37·9% of gonococcal strains, respectively. Patterns I,I', II', and V were uncommon.

Discrimination by different markers and their combinations (LOSs, auxotyping, serotyping, β-lactamase producing strains (PPNG) and antibiotic sensitivity testing) are shown in the table. LOS profiles showed a similar discrimination ability than serotyping or auxotyping. All PPNG Asia$^-$ (4 strains with 4·5 MDa plasmid) were placed into pattern III by LOS. Isolate Asia$^+$ (4·5 MDa and 24·5 MDa plasmids) belonged to pattern IV. The remaining PPNG strains (Africa$^-$ with the 3·2 MDa plasmid) were distributed among LOS banding patterns of intermediate and low molecular weight (I', II, III). None of the penicillinase-producing strains with the same serovar pattern was placed into different groups by SDS-PAGE analysis. A total of 18 isolates were considered multiple antibiotic-resistant strains at low concentrations of antibiotic due to mutation "mtr". Twelve (66·6%) of these 18 multiple antibiotic-resistant strains were included in LOS pattern II. In summary, the use of SDS-PAGE LOS profiles in the differentiation of strains of *N gonorrhoeae*, although is a laborious technique and with difficulties in the interpretation of results by the few differences found among LOS profiles, it may be of great potential use for the study of some epidemiologically important strains for which this phenotypic characteristic may be specific. Characterisation of LOS of *N gonorrhoeae* provides additional information for individual isolates linked to outbreaks of gonococcal infection.

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J M NOGUEIRA*
M A DASI
J J CAMARENA
R GARCIA
J SANZ
J L BARBERA
Department of Microbiology, Faculty of Medicine, University of Valencia, Spain and Department of Clinical Microbiology, Hospital Dr Peset, Valencia, Spain.*

Address correspondence to: Dr Juan J Camarena, Departamento de Microbiología, Facultad de Medicina, Universidad de Valencia. Avda. Blasco Ibáñez 17, 46010 Valencia, Spain.

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